where: m = Percent moisture in the sample.

(6) *Identity.* To about 1 milligram of sample, add 2 milliliters of sulfuric acid; a light-red color is produced when oxytetracycline is present.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[43 FR 11157, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978, as amended at 50 FR 19920, May 13, 1985]

§ 446.67a Sterile oxytetracycline hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile oxytetracycline hydrochloride is [4S - (4α,4aα,5α,5aα,6β,12α)] - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro - 3,5,6,10,12,12a - hexahydroxy - 6 - methyl - 1,11 - dioxo - 2 - naphthacenecarboxamide monohydrochloride. It is produced by the growth of *Streptomyces rimosus.* It is so purified and dried that:

(i) Its potency is not less than 835 micrograms of oxytetracycline per milligram on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) It contains no depressor substances.

(vi) Its loss on drying is not more than 2.0 percent.

(vii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 and not more than 3.0.

(viii) When calculated on an anhydrous basis, its absorptivity at 353 nanometers relative to that of the oxytetracycline working standard similarly treated is 92.5±4.3 percent.

(ix) It gives a positive result to an identity test for oxytetracycline.

(x) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, depressor substances, loss on drying, pH, absorptivity, identity, and crystallinity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Assay for potency by either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Microbiological turbidimetric assay.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of oxytetracycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(ii) *Chemical assay.* Proceed as directed in § 436.320 of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 5 milligrams of oxytetracycline per milliliter.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in § 436.35 of this chapter.

(6) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(7) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(8) *Absorptivity.* Determine the absorbance of the sample and standard solutions in the following manner: Dissolve approximately 50 milligrams each of the sample and standard in 250 milliliters of 0.1*N* hydrochloric acid. Transfer a 10-milliliter aliquot to a 100-milliliter volumetric flask and dilute to volume with 0.1*N* hydrochloric acid. Using a suitable spectrophotometer and 0.1*N* hydrochloric acid as the blank, determine the absorbance of each solution

at 353 nanometers. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{Milligrams of standard}}{\text{Absorbance of standard} \times \text{Milligrams of sample}} \times \text{Potency of standard in micrograms per milligram} \times \frac{10}{100 - m}$$

where: m = Percent moisture in the sample.

(9) *Identity.* To about 1 milligram of sample, add 2 milliliters of sulfuric acid; a light-red color is produced when oxytetracycline is present.

(10) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[43 FR 11158, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978, as amended at 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§ 446.75a Sterile rolitetracycline.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile rolitetracycline is [4S-(4α,4aα,5aα,6β,12aα)] - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro - 3,6,10,12,12a - pentahydroxy - 6 - methyl - 1,11 - dioxo - *N* - (1 - pyrrolidinylmethyl) - 2 - naphthacenecarboxamide. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram on the anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) It contains no depressor substances.

(vi) Its moisture content is not more than 3.0 percent.

(vii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 7 and not more than 9, and such solution is substantially clear.

(viii) It is crystalline.

(ix) When calculated on an anhydrous basis, its absorptivity at 380 nanometers relative to that of the rolitetracycline standard similarly treated is 100±4.4 percent.

(x) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, depressor substances, moisture, pH, crystallinity, absorptivity, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient methyl alcohol to give a solution containing 1 milligram of rolitetracycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of rolitetracycline per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this subchapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this subchapter, using a solution containing 5.0 milligrams of rolitetracycline per milliliter.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in § 436.35 of this subchapter.

(6) *Moisture.* Proceed as directed in § 436.201 of this subchapter.

(7) *pH.* Proceed as directed in § 436.202 of this subchapter, using an aqueous solution containing 10 milligrams per milliliter.